Synovial fluid neutrophil function in RA: the effect of pregnancy associated proteins

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Pregnancy has been associated with remission of symptoms in 75% of women with rheumatoid arthritis (RA).[1 2] This may in part be caused by depression of polymorphonuclear neutrophil (PMN) function, which reduces the degree of synovial fluid (SF) inflammation. This study compared the function of SF and peripheral blood neutrophils from patients with RA and normal subjects, and examined the in vitro effects of pregnancy associated proteins on neutrophil function.

Paired SF and blood samples were obtained from 15 patients with RA (six male, nine female); peripheral blood was obtained from nine normal controls (three male, six female). All patients fulfilled American College of Rheumatology (ACR) criteria for RA.[3] Patients with RA had a mean age of 63 (range 36–81); controls had a mean age of 35 (25–49). Patients with RA had mean disease duration of nine years (1–30); ESR levels mean 50 mm/1st h (SE 6.4) and CRP levels mean 59 mg/l (SE 12.5). In this study, each patient acted as their own control, as both blood PMN and SF PMN were studied. This allowed inclusion of men, and women past child bearing age. All patients with RA were receiving disease modifying drugs; recruitment of subjects not taking drugs is virtually impossible, so this is a caveat in all studies of PMN function studies in RA.

SF samples were pretreated with hyaluronidase (*Streptomyces hyalurolyticus)*. PMN were isolated as previously described.[4] Superoxide anion production (respiratory burst activity) was determined by lucigenin and luminol enhanced chemiluminescence.[5] Respiratory burst occurs when stimulated PMN convert molecular oxygen to toxic oxygen radicals through activation of NADPH-oxidase. This was measured in response to the physiological receptor agonist n-formyl-methionyl-leucyl-phenyalanine (fMLP) and the diacylglycerol analogue phorbol myristate acetate (PMA). We found it was possible to stimulate SF PMN as well as RA blood PMN to initiate a respiratory burst, with greater superoxide anion production than normal blood PMN. SF PMN tended to show greater respiratory burst activity at low agonist concentrations (suggesting priming), although this did not reach statistical significance.

In the joint space PMN undergo degranulation and release their granule contents. Primary and secondary degranulation is demonstrated by increased expression of the integrin CD11b and loss of L-selectin (CD62L).[2] CD11b, CD18, and L-selectin expression were determined by flow cytometry using a direct immunofluorescence method.[6] RA blood PMN and SF PMN both showed a rise in CD11b expression, but not CD18 expression. This indicates that SF PMN have undergone a degree of both primary and secondary degranulation and that was greater than the degranulation in RA blood PMN. SF PMN lost more L-selectin than RA blood PMN; but neither was significantly different from normal blood PMN. Using fMLP increases CD18 and CD11b expression in normal blood PMN and RA blood PMN, but not SF PMN. SF PMN appear to show maximal CD11b expression without added stimulus and the addition of fMLP did not lead to increased loss of L-selectin. Figure 1 shows these results.

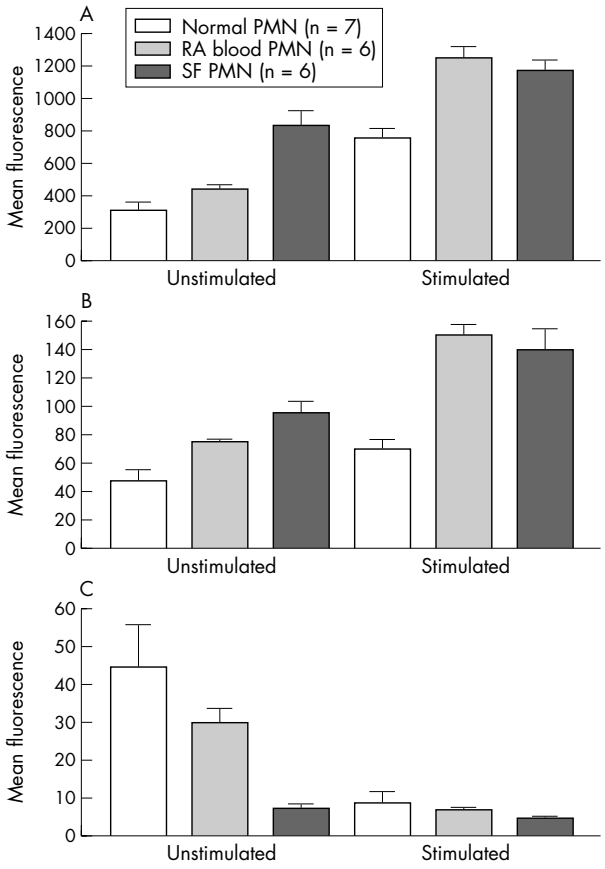

Figure 1 A comparison of adhesion molecule expression for normal blood, RA blood and synovial fluid for (A) CD11b expression, (B) CD18 expression, and (C) CD62L expression. Values are represented as means (standard error).

The effect of pregnancy associated proteins was investigated by adding 10 g/ml β-oestradiol; 50 ng/ml α fetoprotein; 10 μg/ml α_2 macroglobulin; 50 U/ml human chorionic gonadotrophin (hCG)

SF PMN showed reduced extracellular superoxide production on incubation with hCG, and when stimulated with fMLP but not with PMA, suggesting a receptor mediated pathway of activation. This inhibitory effect was not observed in RA blood PMN. Production of SF PMN intracellular superoxide was inhibited by α fetoprotein in stimulated and unstimulated conditions; by α_2 macroglobulin in stimulated conditions; and by β-oestradiol in unstimulated and fMLP stimulated conditions. These proteins may be exerting inhibitory effects on the myeloperoxidase dependent part of the respiratory burst pathway. This confirms a previous observation which highlighted the inhibitory effect of β-oestradiol on PMN superoxide production.[7] Figure 2 shows these effects.

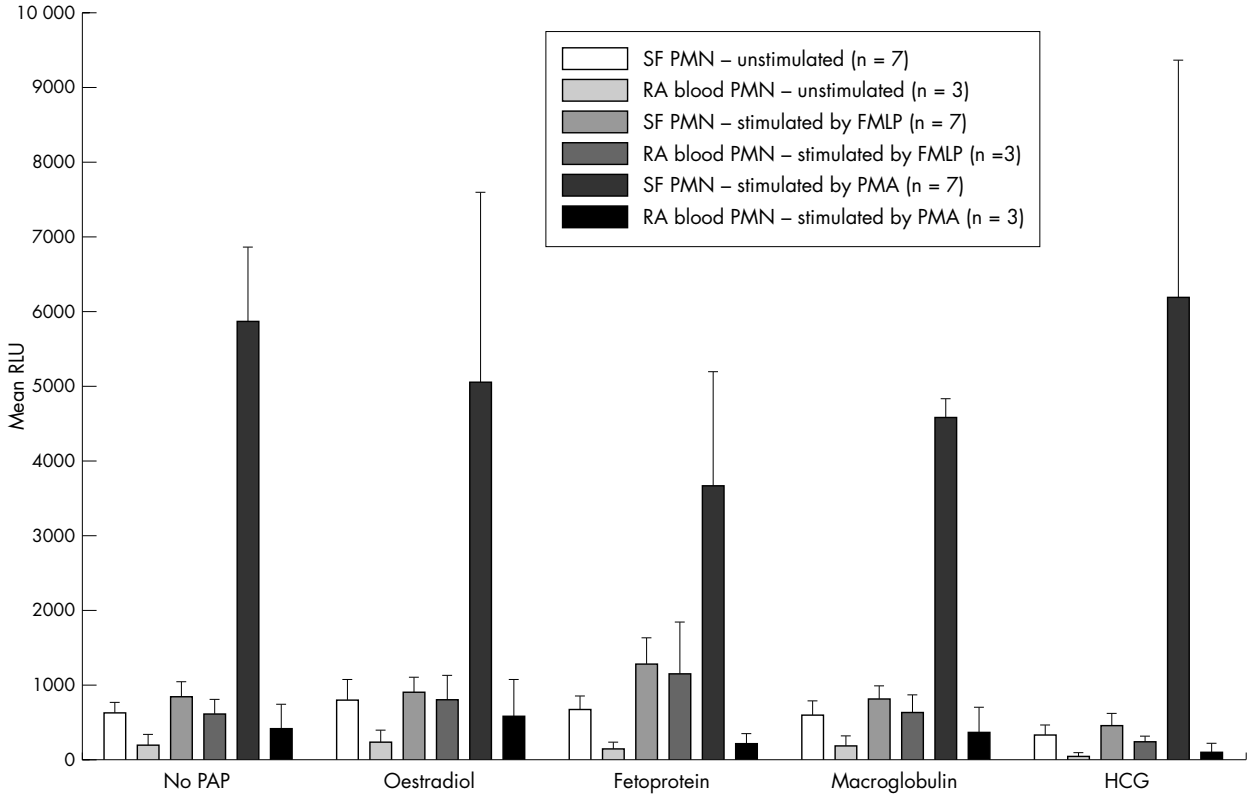

Figure 2 A comparison of the effects of pregnancy associated proteins on lucigenin enhanced chemiluminescence on unstimulated and stimulated SF and RA blood. Pregnancy associated proteins are β-oestradiol, α fetoprotein, α_2 macroglobulin, and hCG. Values are represented as means (standard error).

In summary, we have shown that SF PMN are more responsive than peripheral blood PMN in RA. It would therefore be logical to target the reduction of activation and priming of these cells as a therapeutic approach to reduce inflammation in RA.

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